CERTIFICATE OF HAND DELIVERY

I hereby certify that this paper is being delivered by hand to the U.S. Patent Office in accordance with 37 CFR § 1.6(b), addressed to the Commissioner for Patents & Trademarks, Washington, D.C. 20231, on the date indicated.

[signature] November 18, 2002

Name Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: J. Clark & C. Denning

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Serial No: 09/593,316

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Title: ANIMAL TISSUE FOR XENOTRANSPLANTATION

Art Unit: 1632

Examiner: Quan J. Li, Ph.D.

RESPONSE TO OFFICE ACTION
UNDER 37 CFR § 1.111

Commissioner for Patents and Trademarks
Washington, D.C. 20231

Dear Sir,

This paper is responsive to the most recent Office Action on the merits, dated May 21, 2002 (Paper No. 12), for which a response is due August 21, 2002. Enclosed is a Petition for a three-month extension of time, along with authorization to charge the Deposit Account for the requisite fee. This sets the deadline for responding to November 21, 2002. Accordingly, this paper is timely filed.

Applicant has given careful consideration to the analysis in the Office Action. Please enter the following remarks.

Status of the application:

This is the second Office Action on the merits of the application. The Office Action indicates that Claims 1-7, 13-17, and 27-37 are pending, and that Claims 1-6 and 33-37 are under examination.

In fact, Claim 22 is also still pending in the application. *Therefore, the pending Claims are 1-7, 13-17, 22, and 27-37.*

Group II was rejoined into the group under examination, as indicated in the Office Action mailed November 23, 2001 (Paper No. 7). *Thus, the claims under examination are Claims 1-6, 13-15, and 33-37.* In any event, claims 13-15 are rejoinable upon determination that claim 4 is patentable.

There was a request made in the paper filed to rejoin Group IV (Claim 16) into the application, upon determination that the claims in Group I are patentable. Applicant renews this request, pursuant to MPEP § 821.04. Claim 16 depends from and incorporates the limitations of Claim 1.

Reconsideration and allowance of the application (including all the claims in Groups I, II, and IV) is respectfully requested.

Interview:

Applicant's representative is grateful for the collegial and helpful interview conducted with Examiners Janice Li and James Ketter, at the Patent Office on August 20, 2002. The remarks made below have been structured in accordance with the recommendations made by the Examiners, and are believed to overcome the remaining issues in this application.

Rejections under 35 USC § 112 ¶ 1:

Claims 1-6 and 33-37 stand rejected under § 112 ¶ 1 as not being adequately described or enabled by the specification. Applicant respectfully disagrees for the following reasons.

1. Sheep cells having an inactivated α(1,3)galactosyltransferase allele can readily be produced.

The specification provides for the first time the sheep *α(1,3)galactosyltransferase* (α1,3GT) gene — both in the form of data in the sequence listing, and in the form of the biological deposit (NCIMB Accession No. 41056). Standard methods for using gene sequences to inactivate the gene in living cells are known in the art, and illustrated in the specification. Figures 9-15 provide illustrations of targeting constructs based on the α1,3GT sequence that will inactivate the α1,3GT gene by removing exon sequences. Figure 16 illustrates the successful targeting and deletion of Exon 4, using the p0054 vector. Similar targeting was demonstrated in a *non-isogenic* cell line in Example 5.

The Office Action indicates concern that the specification provides no direct evidence for successful targeting of Exons 8 and 9. Nevertheless, targeting Exons 8 and 9 should be achievable without undue experimentation. In any event, it is not necessary to target Exons 8 and 9 to practice the claimed invention. It is only necessary to eliminate the translation start or remove enough of the gene to prevent the gene product from being functional.

The targeting of Exon 4 (as effected in the working examples), or some other portion of the gene, or various portions in combination should be sufficient for the purpose of inactivating the α1,3GT gene in a sheep cell[1]. The reader may use the constructs provided in the working examples to practice the invention, or alternatively design their own knockout strategy, according to any of the vast technology for making knockouts which is known in the art. Evidence of the level of expertise in the area can be ascertained from the following standard reference books:

- *Gene Knockout Protocols*, by Martin J. Tymms (Editor), Ismail Kola (Editor). 431 pages; Humana Press; 1st edition (January 15, 2001).
- *Laboratory Protocols for Conditional Gene Targeting*, by Raul M. Torres, Ralf Kuhn. Oxford University Press (October 1997).

[1] The enablement requirement is met if the description enables any mode of making and using the claimed invention. *Engel Industries, Inc. v. Lockformer Co.*, 20 USPQ2d 1300 (Fed. Cir. 1991). Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided though broad terminology or illustrative examples. *In re Wright*, 27 USPQ2d 1510 (Fed. Cir. 1993).

- *Homologous Recombination and Gene Targeting*, by John Sedivy. MacMillan Pub Co. (November 1991).
- *Gene Targeting: A Practical Approach*, by Alexandra L. Joyner (Editor). Oxford University Press; ISBN: 019963792X; 2nd edition (February 15, 2000).
- *The Gene Knockout Factsbook* (2-Volume Set), by Tak W. Mak (Editor), Josef Penninger (Editor), John Roder (Editor), Janet Rossant (Editor), Mary Saunders. 1140 pages; Academic Press; 1st edition (November 15, 1998).
- *Gene Targeting Protocols (Methods in Molecular Biology, Vol 133)*, by Eric B. Kmiec (Editor), Dieter C. Gruenert (Editor). Humana Press (January 15, 2000).

2. *Animals having an inactivated α1,3GT allele can readily be made from inactivated donor cells by nuclear transfer*

Several methods are available for making genetically modified animals from genetically altered cells. The specification indicates that animals can be cloned from a suitable donor cell by nuclear transfer. This is proven technology that created Dolly the sheep. The nuclear transfer method has been fully described and enabled for the advancement of the art, as indicated by issuance of U.S. patents 6,147,276 and 6,252,133 (Campbell & Wilmut, Roslin Institute).

There is no reason to believe that genetically altering the donor cell would affect its suitability as a nuclear donor. To the contrary. A number of published experiments confirm that cloned animals may readily be made from genetically altered cells according to the Campbell & Wilmut method.

- Denning et al. (Nat. Biotechnol 19:559, 2001) describe the deletion of the alpha(1,3)galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. Eight pregnancies were maintained to term and four PrP-/+ lambs were born.
- Uchida et al. (Transgenic Research 10:577, 2001) report the production of transgenic miniature pigs by pronuclear microinjection. The huntington gene cloned from miniature pig, was linked to rat enolase promoter, and injected into pronucleus of fertilized eggs. Several of the offspring were determined to have the transgene by PCR and Southern analysis.
- Bondoli et al. (Molec. Repro. Dev. 60:189, 2001) report cloned pigs generated from cultured skin fibroblasts derived from a boar with an H-transferase transgene. Two healthy piglets resulted from nuclear transfer by fusion of fibroblasts that had been extensively cultured with enucleated oocytes.

- Lai et al. (Molec. Repro. Dev. 62:300, 2002) report a transgenic pig expressing green fluorescence protein. Fetal-derived fibroblast cells were transduced with the GFP gene, and then cloned into porcine oocytes. A healthy transgenic piglet was obtained that expressed GFP.
- Lai et al. (Science 295:1089, 2002) report production of α(1,3)galactosyltransferase knockout pigs by nuclear transfer cloning. The pigs were produced by nuclear transfer, using clonal fetal fibroblast cell lines as nuclear donors.
- Dai et al. (Nature Biotech 20:251, 2002) also report production of α(1,3)galactosyltransferase knockout pigs by nuclear transfer cloning. The pig α1,3GT gene was disrupted in both male and female porcine primary fetal fibroblasts, which were then used for nuclear transfer. Six clonal fetal piglets were obtained, of which five were normal weight and apparently healthy. Southern blot analysis confirmed that the five piglets contained one disrupted α1,3GT allele.

The last two references are of particular interest, because they illustrate that α1,3GT knockouts can readily be made in another ungulate species (the pig), using the nuclear transfer method, and enabled by possession of the corresponding α1,3GT gene.

3. Animals that are homozygous for inactivated α1,3GT can readily be produced

The specification refers to U.S. Patent 5,589,369, which provides a method of making diploid mammalian cells homozygous for disrupted target loci (Claim 1 of the '369 patent). Homozygous knockout cells can be made into homozygous animals by nuclear transfer by the method of Campbell & Wilmut. Currently, a more typical practice is to use a nuclear donor cell with α1,3GT inactivated on one allele, produce a heterozygous knockout animal, and then interbreed such animals to produce a homozygous knockout. This is described in the specification on page 41, along with additional alternatives.

Since these technologies are in wide-spread general use, the only relevant question in relation to the invention claimed in this application is whether knocking out both α1,3GT alleles would somehow compromise the viability of the animal.

In fact, we know this not to be the case. Humans and other Catarrhine primates are exceptions amongst mammalian species as not having an expressed α1,3GT gene. We seem to get along quite well without it. Furthermore, U.S. Patent 5,849,991 (Cols. 48-57) describes the isolation of the mouse α1,3GT gene, and using it to make homozygous α1,3GT knockout mice. Such mice have been used

extensively in labs around the world for immunological and transplant studies, and have the usual features of animals of the murine species.

Based on the precedents of humans, other Catarrhine primates, and homozygous knockout mice, there is no reason to believe that homozygous knockout sheep would not resemble normal sheep in most characteristics except expression of the Galα(1,3)Gal phenotype.

4. Cells from homozygous knockout animals will have cells and tissues devoid of the Galα(1,3)Gal xenoantigen

As described in the specification, the α1,3GT gene is uniquely responsible for forming the Galα(1,3)Gal xenoantigen in non-Catarrhine mammals. An animal that is homozygous for inactivation of the α1,3GT gene would therefore be devoid of the Galα(1,3)Gal antigen, having no other way of making it.

U.S. Patent 5,849,991 (referred to earlier) describes homozygous α1,3GT knockout mice. Peripheral blood monocytes and splenocytes from the homozygous knockouts were analyzed for presence of the Galα(1,3)Gal antigen using the IB4 lectin, in a manner comparable to what is described in the specification of the present application on pages 41-43. Wild-type mice showed high degree of staining, while knockout mice showed minimal staining, confirming that the tissue was devoid of the Galα(1,3)Gal antigen (U.S. 5,849,991, Cols. 48-52). As expected, since Galα(1,3)Gal is not a self-antigen in these mice, they form naturally occurring antibody against it, as do humans (Chong et al., Transpl Immunol 8:129-37, 2000).

In summary, techniques suitable for preparing α1,3GT knockout animals are generally known in the art, and referenced in the specification[2]. It has not previously been possible to make α1,3GT knockout sheep, simply because the sheep α1,3GT gene was not previously available. Now that the sheep α1,3GT gene has been discovered and characterized, it is now straight forward to produce sheep tissue which is devoid of Galα(1,3)Gal, or which has been inactivated for the α1,3GT gene on one or both alleles, using techniques already proven to be effective in the mouse and the pig.

Accordingly, the claimed invention is fully described and enabled in the specification, and deserves to be issued as a patent. Withdrawal of the rejections under 35 USC § 112 ¶ 1 is respectfully requested.

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[2] A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991).